IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	**09/707,117**
Applicants	:	**Jon A. Wolff, Vladimir Budker**
Filed	:	**11/06/2000**
Art Unit	:	**1632**
Examiner	:	**Wilson, Michael C.**
Docket No.	:	**Mirus.018.02**

For: **Intravascular Delivery of Nucleic Acid**

Commissioner of Patents
PO Box 1450
Alexandria, VA 2231-1450

APPELLANT'S AMENDED BRIEF under 37 CFR 41.37

(i) Real party in interest:

The real parties in interest are: Jon A. Wolff and Vladimir Budker and, by assignment, Mirus Corporation, which has changed its name to Mirus Bio Corporation under the laws of the State of Delaware and is located at 505 South Rosa Road, Madison, WI 53719.

(ii) Related appeals and interferences:

There are no interferences known to appellant, the appellant's legal representative, or assignee which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(iii) Status of Claims:

Claims 1-3, 6, 7, 11, 12, 16-20, 24, 25, 28-31, 34-36 and 39-42 have been rejected and are hereby appealed.

Claims 4, 5, 8-10, 13-15, 21-23, 26, 27, 32, 33, 37 and 38 have been canceled.

(iv) Status of amendments:

No Amendments have been filed subsequent to the final rejection.

(v) Summary of Claimed Subject Matter:

The claimed subject matter is a process for delivering a polynucleotide to a cell in a mammalian limb. The inventors injected polynucleotides into Rhesus macaque monkey limb blood vessels, and limbs of other mammals, and caused the polynucleotide to be delivered to limb extravascular cells. Importantly, for both arm and leg injections, blood flow during the injection was impeded by a cuff (tourniquet) on the surface of the skin and/or fur, surrounding the arm or leg (Example 1 beginning on page 23, line 11; Example 3 beginning on page 25, line 15, and Example 10 beginning on page 32 line 9). This aspect is essential. Applying a cuff is non-invasive relative to prior art methods. According to the prior art, to clamp off a blood vessel, surgery must be performed and the claim applied while the surgical wound is open. In contrast, a cuff is simply wrapped around the limb and pressure applied, in the same way a sphygmomanometer cuff is applied to measure blood pressure.

A cuff surrounding the limb is defined in the specification as device placed external to the mammal's skin and which applies pressure against the limb to constrict blood vessels in an area underneath the cuff in amount sufficient to impede blood from flowing at a normal rate. Disclosed exemplary cuffs include a sphygmomanometer and a tourniquet, both of which are commonly used devises for occluding blood flow in a limb (page 5, lines 13-24).

Injection of a polynucleotide-containing solution into a limb blood vessel combined with occluding blood flow using the cuff around the limb results in increased permeability of blood vessels and delivery of polynucleotides to extravascular cells in the limb (page 2 lines 26-32; page 4 line 27 to page 25 line 3; and, page 17 line 8 to page 18 line 6). High levels of expression of delivered genes were found in muscle groups throughout the limb distal to the blood vessel occlusion (Table A, beginning on page 26, line 15; and, Table B, beginning on page 27). Expression levels in monkeys and rats were similar (Examples 7-10 beginning on page 30, line 19). Example 8 on page 31 is specific to expression of a therapeutic gene.

(vi) Grounds of rejection to be reviewed on appeal:

Whether claims 1-3, 6, 7, 11, 12, 16-20, 24, 25, 28-31, 34-36 and 39-42 are unpatentable under 35 U.S.C. 112, first paragraph, as containing new matter.

Whether claims 1-3, 6, 7, 11, 12, 16-20, 24, 25, 28-31, 34-36 and 39-42 are unpatentable under 35 U.S.C. 112, first paragraph, for lack of enablement.

Whether claims 1-3, 6, 7, 11, 12, 16-20, 24, 25, 28-31, 34-36 and 39-42 are unpatentable under 35 U.S.C. 112, second paragraph, as being indefinite.

Whether claim 39 is unpatentable under 35 U.S.C. 102 as being anticipated by U.S. Patent 6,495,131 and Von Der Leyen et al. 1999 (Human Gene Therapy Vol. 10 p. 2355-2364).

Whether claims 1-3, 6, 11, 12, 16, 17, 28, 30, 31, 34-36 and 39-42 are unpatentable under 35 U.S.C. 103 as being unpatentable over Budker et al. 1998 (Gene Ther. Vol. 5 p. 272-276) or U.S. Patent 6,265,387 in view of Milas et al. 1997 (Clin. Cancer Res. Vol. 3 p. 2197-2203).

Whether claims 1-3, 6, 7, 11, 12, 16-20, 24, 25, 28-31, 34-36 and 39-42 are unpatentable under the judicially created doctrine of obviousness-type double patenting over U.S. Patent 6,265,387 or U.S. Patent, 6,327,616 in view of Milas et al. 1997 (Clin. Cancer Res. Vol. 3 p. 2197-2203).

(vii) Argument:

(i) Rejection of the Claims under 35 U.S.C. 112, first paragraph.

The initial rejection on page 3 of the Office Action states that the phrase "non-invasive" in claims 1 and 39 does not have support in the specification and is new matter. This rejection refers to claim 1, step a) "*applying non-invasive external pressure against the skin of a limb of the mammal such that blood flow to and from the limb is impeded.*" The Action states on page 3: "The specification does not implicitly support applying pressure without inserting an instrument or device into the body through the skin."

Although not implicit, Applicants' specification explicitly states on page 5, lines 13-15: "The term cuff means a device for impeding blood flow through mammalian internal blood vessels. However, for purposes of the claims, cuff refers specifically to a device applied exterior to the mammal's skin and touches the skin in a non-invasive manner." The statement clearly defines the application of non-invasive pressure to the outer skin. What is unclear is why this definition does not provide support.

Page 4 of the Action states that the examiner provided (from the previous Action) two definitions of invasive: "1) denoting a procedure requiring insertion of an instrument or device into the body through the skin or a body oroifice or 2) to affect injuriously and progressively. The specification does not implicitly support applying pressure without inserting an instrument or device into the body through the skin." Based on those statements, Applicants believe that the Examiner does not understand the invention. It seems that the examiner is confusing the non-invasive application of the cuff with the invasive insertion of the needle which provides the injection. Applicants have been explicit in their description of the non-invasive pressure to the mammalian skin.

The action states on page 4 that applying a cuff as described in the specification has a much smaller scope that applying non-invasive pressure. It is well known in the art that cuffs such a tourniquets and sphygmomanometer apply pressure. Support for applying pressure to a limb to occlude blood flow is provided in the specification on page 3 lines 8-15. The mechanism of action of a cuff and the desired outcome is described in detail on page 5 lines 13-19. Applicants' provide unambiguous support for "applying non-invasive external pressure against the skin of a limb of the mammal such that blood flow to and from the limb is impeded" as claimed.

The Action states, in the last paragraph on page 4, that the term "solution" is new matter because no support is provided for the breadth of any "solution". Support for the term solution is provided in the specification in example 1 (starting on page 23), example 8 (starting on page 31), example 9 (starting on page 31), and example 10 (starting on page 32). In each of these examples it is explicitly stated that DNA in a solution is injected into the limb vessel.

The Action states, in the first paragraph on page 5, that the term "expressible sequence" does not have support in the specification. By common usage, the term expressible sequence means that the sequence is able to be expressed. That a sequence is to be expressed is supported throughout the specification (see page 1 lines 20-24, page 4 line 29, page 5 lines 5-8, page 14 line 32, and page 15 lines 21-22). The specification also explicitly states that the transferred nucleic acid may contain an expression cassette (page 2 lines 6-7). Expression cassette is defined starting on page 6 line 28 of the specification as a "nucleic acid which is capable of expressing protein", i.e. an expressible sequence.

On page 7, the rejection under 35 U.S.C. 112 states that the Specification is not enabling for delivery of a viral vector to limb skeletal muscle cells. Delivery of viral vectors is supported in the Specification on page 15 lines 9-19. Applicants have also provided, in a declaration under 37 CFR 1.132 filed May 9, 2003, examples of viral delivery using methods within the scope of the specification.

Starting in the last paragraph on page 7 the Action states that vector targeting is unpredictable and inefficient according to some prior art references. Applicants point out that a novel aspect of their invention is derived from their ability to deliver nucleic acids to a "desired tissue" such as limb muscles. This novelty has been demonstrated in each of the Examples written in the Specification. Applicants respectfully remind the Examiner that each of these references listed, Miller (1995), Deonarain (1998), Verna (1997) and Crystal (1995), was published prior to the filing date of Applicants' Specification and none contemplates the method described by Applicants. Prior art that makes an attempt to target polynucleotides to desired tissues cannot be used to refute subsequent art that effectuates targeted delivery of polynucleotides. Applicants believe that they have significantly added to the art. Therefore, it is unclear how the cited prior art, which couldn't target tissues, applies to Applicants' process which can.

In the first full paragraph on page 7, the Action states that Milas taught applying pressure and injecting an adenovirus distal to the applied pressure as encompassed by steps a and b of claims 1 and 39. In the same paragraph, the Examiner states, "Milas did not obtain protein expression in skeletal muscle cells distal to the applied pressure using adenoviral vector." Applicants agree with this statement.

The Examiner further states, "Milas taught that applying pressure non-invasively and injected an adenovirus distal to the applied pressure as encompassed by steps a and b of claims 1 and 39." This statement is inaccurate since steps a and b of claims 1 and 39 require impeding blood flow to and from the limb. Milas et al., in contrast, clearly state, "the perfusion pump was positioned below the plane of the animal to allow venous outflow" (page 2199 column 1); "the perfusate entered via the femoral artery and returned via the femoral vein" (page 2199 column 1); "cannulation of the femoral vein with resultant brisk outflow is critical for the success of the procedure" (2202, paragraph 1). Applicants' claims do not suggest an

invasive perfusion technique as put forth in Milas et al. Furthermore, Applicants' believe that the differences between the process as taught by Milas and the Applicants invention account for the lack of delivery by Milas and the effective delivery demonstrated using the Applicants' process.

The Action states in the last paragraph on page 7 that "the method claimed is not limited to applying pressure non-invasively without applying pressure invasively." Applicants are unable to respond to this statement. The claim specifically states pressure is applied non-invasively to impede blood flow.

The Action further states (page 7 bridging to page 8) that "the claims encompass blocking blood flow using a tourniquet while allowing some blood to flow out of the limb using a perfusion pump as taught by Milas and are not limited to blocking blood flow in and out of the limb without allowing any blood to outflow from the limb." This statement is incorrect. Claims 1 and 39 state that blood flow to and from the limb is impeded. Applicants specifically teach that not occluding blood flow results in no delivery (page 30 lines 23-24). In contrast, Milas taught perfusing a limb by inserting a solution containing adenovirus into an artery while allowing brisk outflow through a vein. As noted above, Milas notes that providing brisk outflow is critical to their procedure (ref. page 2202, column 1). This difference is one reason why Milas failed to observe delivery to skeletal muscle cells in the limb.

The Action further states, beginning on page 9 last paragraph, that Ye et al. confirm that adenoviral vectors would not be expected to cause expression in skeletal muscle cells using the steps claims. The Action states Ye taught administering adenoviral particles to the portal vein/artery occluded with clamps. This statement is incorrect. Ye taught injection of adenovirus into the retro-orbital venous plexus, a vessel of the eye. Ye also taught clamping of the hepatic artery and the portal vein to prevent blood circulation, and thus the adenovirus, from reaching the liver. In contrast, Applicants teach using non-invasive pressure to impede blood flow to and from the limb and injecting a polynucleotide into a blood vessel in a limb distal to the occlusion. Therefore, it is expected that the method taught by Ye would provide different results than the method taught by the Applicants.

On page 10-12, the Action states the example of viral delivery presented in the declaration under 37 CFR 1.132 is not supported in the specification. The Examiner contends that the specification suggests using either papaverine or collagenase, but not both. The specification clearly states that an agent may be used to further increase permeability of blood vessels (page 5 lines 26-28, page 16 line 19 to page 17 line 7). Applicants further describe a number of different agents that work by a variety of mechanisms which may be used to further increase vessel permeability: hypertonic solutions, molecules that interact with a specific receptor or enzyme or protein within a vascular cell, molecules that can increase permeability by changing the extracellular connective material, and chelators. Applicants state that an agent may be used to further enhance permeability. The statement does not imply that only one of a provided list of possible agents may be used.

The Examiner postulates that the use of papaverine together with collagenase may have a synergistic effect that is essential for adenoviral delivery, but does not provide any reasoning. The Examiner further argues that it would not have been readily apparent to one skilled in the art to use the specific combination of papaverine and an enzyme together. Applicants respectfully disagree. One skilled in the art would consider the possibility of success in combining multiple agents that work by different mechanisms.

The Action states on page 10 bridging to page 11 that the concentration of $5x10^8$ adenoviral particles in 10 ml saline, as described in the declaration, is not taught anywhere in the specification. The Action further states, on page 12, that one of skill in the art would have known that injecting $5x10^8$ adenoviral particles described in the declaration was essential to the invention.

The exact number of DNA molecules or viral particles to inject is a determination of dosage. The MPEP, 2164.01(c), states that it is not necessary to specify the dosage if it is known to one skilled in the art that such information could be obtained without undue experimentation as is the case here. The exact number of adenoviral particles injected does not determine whether the claimed invention will work for its intended purpose. Nevertheless, one can estimate that the number of plasmid molecules injected in example 8 of the specification would be approximately $6x10^{13}$ plasmid molecules ($500x10^{-6}$ g ÷ ~$5.2x10^6$ g/mole (molecular weight of an 8 kb plasmid) × $6.02x10^{23}$ molecules/mole). This number is well above the $5x10^8$ adenoviral genomes injected in the declaration supporting example.

The Action states (page 11) the 1.32 declaration does not correlate to the claims because the claims are not limited to delivering adenovirus, delivering adenovirus in combination with papaverine and collagenase, or to injecting within 10 sec. However, Applicants believe that the methods used in the declaration are reasonably supported in the specification and encompassed by the claims. *(MPEP 2164.01(b) As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. 112 is satisfied.)*

The Action states on page 12 that the specification does not correlate the volumes required to deliver non-viral vectors with the volume required to delivery viral vectors. Injection of 10 mls in 10 sec into a rat leg artery is supported in the specification (page 31 lines 11-12) and is effective for delivery of both viral vectors and non-viral vectors to rat hindlimb. The specification does not provide different volumes for viral and non-viral vectors because different volumes are not required. Applicants teach that polynucleotide delivery is increased by increasing the permeability of the tissue's blood vessel and permeability is increased by: increasing the intravascular hydrostatic pressure, delivering the injection fluid rapidly, using a large injection volume, and inhibiting fluid flow out of the limb (page 4 lines 27-33).

(ii) Rejection of the claims under 35 U.S.C. 112, second paragraph (Office Action page 13):
The claims have been rejected under 35 U.S.C. 112, second paragraph, as being indefinite. On page 13 of the Action, the Examiner states that "skeletal muscle cell of a mammal" in the preamble is not commensurate in scope with "skeletal muscle cell in that limb distal to the applied pressure" in claim 1. The Action contends that it is unclear if the polynucleotide is delivered to any skeletal muscle cell or to just skeletal muscle cells in a limb distal to an applied pressure.

However, a skeletal muscle cell as stated in the preamble includes the more narrowly claimed skeletal limb muscle cell. The preamble cannot be read without considering the limitations in the rest of the claim. Applicants' believe that their plainly stated claim is unambiguous in claiming delivery to skeletal muscle cells which are located in a limb distal to an applied occlusion.

The Action further states that the phrase "the skeletal muscle cell in the limb distal to the applied pressure" in step c lacks antecedent basis. The term "skeletal muscle cell" is introduced in the preamble, and the terms limb and pressure are introduced in step a.

In the first paragraph on page 14, claim 1 has been rejected as indefinite because the phrase "the polynucleotide encoding a protein operably linked to a promoter in a solution into a vessel" lacks proper antecedent basis. Applicants respectfully disagree. The polynucleotide has proper antecedent basis in the preamble of the claim. Citing that the polynucleotide encodes a protein and is linked to a promoter merely serves to further define the properties of the polynucleotide.

Claims 1 and 39 have been rejected as indefinite for the phrases "applying non-invasive external pressure" and "applying pressure non-invasively" respectively. Applicants respectfully disagree for the reasons already stated in response to the previous 112, first paragraph, rejections.

In the first full paragraph on page 15, claim 39 has been rejected as being unclear whether "use of the limb" is limited to function of the limb or frequency of use of the limb. The Examiner admits that the specification supports full function of the limb and no discomfort, but not frequency of use. The Examiner postulates that "the monkeys must have had diminished use of the limb right after surgery". No rationale or explanation is provided. Furthermore, this assumption appears to be without basis since the specification teaches that the monkeys "did not appear to be in any discomfort beyond that of normal surgical recovery" (page 25 lines 22-24). Claim 39 cites that the delivery process does not diminish subsequent use of the limb.

In the first full paragraph on page 16, claim 39 has been rejected as indefinite because the phrase "the polynucleotide encoding an expressible sequence operable linked to a promoter in a solution" lacks proper antecedent basis. Applicants respectfully disagree. The polynucleotide has proper antecedent basis in the preamble of the claim. Citing that the polynucleotide encodes an expressible sequence and is linked to a promoter merely serves to further define the properties of the polynucleotide.

In paragraph 2 on page 16, the Action states "The phrase 'the skeletal muscle cell of the limb distal to the applied pressure' in step b) of claim 39." Applicants suspect that this fragment is a leftover from another Action and have no response.

(iii) Rejection of the claims under 35 U.S.C. 102:

Claim 39 has been rejected under 35 U.S.C. 102(e) as being anticipated by Draijer van der Kaaden (U.S. Patent 6,495,131; hereafter '131). The Examiner states, on page 16 of the Action, that '131 taught adenovirus was perfused through the leg using a pump. Applicants agree. The Examiner also states that '131 taught a tourniquet impeded blood flow into and out of the blood vessels in the leg. This statement is inaccurate.

The tourniquet, as used by '131, was used to restrict the adenovirus to certain tissues or organs to avoid unnecessary infections (column 6 lines 25-30). It did not prevent blood flow out of the limb, since '131 taught that perfusion was performed by creating a closed circuit and repassing the adenovirus through the isolated tissue (column 10 lines 45-55). Specifically, a closed circuit was formed between the femoral artery and the femoral vein (column 11 lines 26-27, example 3 in column 17).

The Action states in the first paragraph on page 17 that the claims encompass using a tourniquet in combination with the perfusion pump described by '131. Applicants disagree and, moreover, do not understand how the claims can be interpreted to encompass forming a vascular closed circuit through which fluid is circulated using a perfusion pump as described in the '131 patent.

In the third paragraph on page 17, the Action states that the method of '131 results in delivery of adenovirus to skeletal muscle as evidenced by Table II. The Examiner specifically points out that perfusion for 30 minutes gave greater expression in skeletal muscle than perfusion for 5 minutes. However, Applicants respectfully point out that the inventors of '131 clearly state: "administration via the circulation mainly transduces endothelial cells of the tumor vasculature" (column 12 lines 20-21), "gene transfer into tissues other than the tumor hardly occurs using either method" (isolated limb perfusion or direct tumor injection, column 12 lines 23-24), staining of tissues showed color (i.e., expression product) "was restricted to areas directly adjacent to the blood vessels of the tumor" and not even in the tumor itself

(column 18 lines 47-50), and "no high uptake of (adenovirus) by the liver or skeletal muscle of the isolated limb after ILP or intra-tumor injection (column 18 lines 19-22).

Claim 39 has been rejected under 35 U.S.C. 102(a) as being anticipated by Von der Leyen (Human Gene Therapy Vol. 10, pg 2355-2364). The Action states, in the last paragraph on page 17, that von der Leyen taught DNA delivery into the carotid artery while applying a sphygmomanometer to the skin of the limb.

Applicants can find no support in Von der Leyen for the placement of a sphygmomanometer to the skin of the limb or to impeding blood flow to and from a limb using a sphygmomanometer. It is the Applicants' opinion that the Examiner mistakenly assumes that because Von der Leyen et al. state a sphygmomanometer was used, that it must have been used to impede blood flow to and from a limb (also last paragraph on page 18).

Additionally, the Action states that Applicants' argument regarding the angioplasty monometer is irrelevant. Applicants strongly disagree. Von der Leyen et al. state that for pressures between 0 and 300 mm Hg, a sphygmomanometer was used to monitor pressure, and for pressures between 300 and 760 mm Hg, a percutaneous translumenal coronary angioplasty (PCTA) monometer was used. If a PCTA manometer can substitute for a sphygmomanometer in the procedure, then Von der Leyen could not have been using the sphygmomanometer to occlude blood flow to a limb.

The Action admits (Action: page 17 bridging to page 18) that Von der Leyen did not state obtaining delivery to skeletal muscle. However, it is the Examiner's contention that Von der Leyen implicitly taught delivery to skeletal muscle, because they "forced DNA through the blood vessel wall (pg 2364, col. 1, line 14)". Applicants respectfully point out that this line describes previous studies which studied convective forces resulting from pressure gradients. The authors state, in the same paragraph, that their procedure induces a nongradient pressurized environment and that there are no convective forces except during the initial pressurization. Von der Leyen et al. only describe transfer of DNA to cells of the vessel lumen, cells of the vessel neointima, media, and adventitia layers (see pg. 2360 col. 1 and Fig. 2). Von der Leyen do not describe delivery to any cell other than a cell of a blood vessel wall or suggest that their method can be used to delivery DNA to cells outside of a blood vessel.

In the first paragraph on page 19, the Action states that Von der Leyen et al. may have isolated the artery during the balloon angioplasty, but did not do so during the transfection. Applicants respectfully disagree. The following statements are taken directly from Von der Leyen et al: "After isolation of either previously injured or normal uninjured carotid artery ..." (first sentence of "*Transfection Procedure*" page 2356); and, "For normal uninjured carotid artery, gene transfer was performed immediately after the isolation of the vessel." (page 2357, col. 1, line 7).

On page 19, the Action states that in the method taught by Von der Leyen et al., the space between the clamp and the sheath or the ligature and the sheath would allow delivery of adenovirus to skeletal muscle surrounding the artery. The Action further states that adenovirus may have leaked out from the sheath into surrounding skeletal muscle. Applicants note that Von der Leyen did not teach the use of adenovirus. Finally, the Action states that the vessels were damaged by angioplasty and thus inherently had leakage into the surrounding skeletal muscle. Applicants can find no support for these statements in the Von der Leyen et al. reference.

Von der Leyen teach a method for delivery of nucleic acid to cells in an isolated carotid artery segment protected by a sheath. Applicants are claiming a process for delivery to limb skeletal muscles cells distal to an occlusion in the limb by injecting polynucleotides into a vessel in the limb distal to the occlusion. However, the carotid artery is not in a limb, it is in the neck, and skeletal muscle surrounding the carotid artery can not be located in a limb distal to the placement of a sphygmomanometer on a limb. Therefore, Von der Leyen cannot anticipate Applicants' claims.

(iv) Rejection of the claims under 35 U.S.C. 103:

Claims 1-3, 6, 11, 12, 16, 17, 28, 31, 34-36 and 39-42 have been rejected under 35 U.S.C. 103 as being unpatentable over Budker et al. 1998 (Gene Ther. Vol. 5 p. 272-276) in view of Milas et al. 1997 (Clin. Cancer Res. Vol. 3 p. 2197-2203).

The Action argues, page 20-22, that it would have been obvious to combine the tourniquet as taught by Milas with the Budker delivery method. However, Milas teaches the use of a surgically placed tourniquet (underneath the inguinal ligament, page 2198, col. 2) for the

purpose of prevention adenoviral infection of cells outside the limb, together with perfusion, in which brisk outflow was critical, to delivery to tumor cells in the limb. In contrast, Applicants claim non-invasive occlusion to aid in delivery of polynucleotide to limb skeletal muscles. As the Examiner admits, the method of Milas fails to delivery adenovirus to limb skeletal muscle cells. Therefore, it could not have been obvious to combine a method that explicitly fails to deliver virus to skeletal muscle cells with the surgically invasive occlusion method taught by Budker.

In the second paragraph on page 20, the Action states administering collagenase as taught by Budker is equivalent to applying immunosuppression. Yet, Budker did not teach immunosuppression as Applicants addressed in a declaration filed under 36 CFR 1.132 in a reply entered on Jan. 28, 2005. The Declaration provides evidence that injecting collagenase is not equivalent applying immunosuppression.

As discussed above, neither Milas nor Budker taught non-invasive occlusion of blood flow to and from a limb or applying immunosupression. Therefore, Budker combined with Milas did not teach all the limitations of the Applicants' claims.

In the second full paragraph on page 22, claims 1-3, 6, 11, 12, 16, 17, 24, 25, 28-31, 34-36 and 39-42 have been rejected under 35 U.S.C. 103 as being unpatentable over U.S. Patent 6,265,387 in view of Milas et al. 1997 (Clin. Cancer Res. Vol. 3 p. 2197-2203).

The action states, on page 23, that this rejection was not addressed by the Applicants' prior response. However, Applicants addressed this rejection together with the 103 rejection over Budker, since both rejections rely on Milas et al. As discussed above in response to the §112 and §103 rejections, and as stated in the Office Action (page 22, last paragraph), Milas taught an invasive surgically placed tourniquet under the inguinal ligament. Milas did not teach non-invasively occluding blood flow to and from a limb as claimed by the Applicants.

(v) Obviousness-type double patenting:

In the Office Action on page 23, claims 1-3, 6, 11, 12, 17, 24, 25, 28-31, 34-36 and 39-42 were rejected for obviousness-type double patenting as being unpatentable over claim 1 of U.S. Patent No. 6,265,387 in view of Milas et al.

Claim 1 in the '387 patent reads as follows:

1. A method for delivering naked plasmid DNA into a hepatocyte of a mammal comprising:
   a) injecting a composition into the bile duct of a mammal, said composition consisting of naked plasmid DNA encoding a protein operably linked to a promoter and a pharmacologically acceptable solution; and
   b) increasing the permeability of said bile duct to allow the composition through the bile duct wall and into the liver of the mammal such that said plasmid DNA is delivered to a hepatocyte of the liver, and said hepatocyte expresses said protein to a detectable level.

As the Action states, in the last paragraph on page 23, '387 claims delivering naked DNA to liver via injection into bile duct and did not claim delivery to skeletal muscle. On page 24, the Action states that because '387 disclosed injection into a clamped femoral artery, injection into a femoral vein is an obvious variation of injection into a bile duct and one would have been motivated to use clamps in increase permeability in light of the '387 disclosure. The Action further states, on page 25, that it would have been obvious in light on Milas to substitute a tourniquet in place of clamps.

MPEP 804 states, "When considering whether the invention defined in a claim of an application is an obvious variation of the invention defined in the claim of a patent, the disclosure of the patent may not be used as prior art." and "... a double patenting rejection must rely on a comparison with the claims in an issued or to be issued patent." Clamping or occlusion is not claimed in '387. Additionally, injection of polynucleotides is patentably distinct from injection of naked plasmid DNA. Injection into a vessel in a limb is patentably distinct from injection into a bile duct. Delivery to limb skeletal muscle cells is patentably distinct from delivery to hepatocytes of the liver.

On page 26 of the Office Action, claims 1-3, 6, 7, 11, 12, 16-20, 24, 25, 28-31, 34-36 and 39-42 were rejected for obviousness-type double patenting over claims 1-4 of U.S. Patent No. 6,627,616 in view of the disclosure of Milas.

Claims 1-4 of '616 read as follows:

1. *A process for delivering naked polynucleotides to an extravascular cell of a mammal, comprising: a) injecting the naked polynucleotides into a blood vessel lumen, in vivo; b) increasing permeability in the blood vessel; and, c) delivering the naked polynucleotides to extravascular cells outside of the blood vessel via the increased permeability.*
2. *The process of claim 1, wherein increasing the permeability of the vessel consists of increasing pressure inside the vessel.*
3. *The process of claim 1, wherein the permeability of the vessel is increased by inserting papaverine into the vessel with the naked polynucleotide.*
4. *A process for delivering naked polynucleotides to an extravascular cell of a mammal, comprising: injecting the naked polynucleotides into a tail vein, increasing pressure inside the tail vein and delivering the naked polynucleotides to an extravascular cell not within the tail.*

In contrast to the '616 claims and disclosure, Applicants have described non-invasive methods for delivering polynucleotides to skeletal muscle cells in a mammalian limb by applying a device for impeding blood flow to the surface of the skin of a limb (exterior occlusion). The finding that polynucleotide delivery to limb skeletal muscle cells can be performed using a non-invasive procedure is a significant and non-obvious advancement over the prior art. As the Action notes (first paragraph page 27) the combined teachings of the claims and the disclosure of '616 did not teach using a tourniquet (i.e. non-invasive occlusion of fluid flow to a limb). It is the Examiner's opinion (see pages 27-28 of the Action), however, that one would have been motivated to combine the teachings of Milas with the teachings of Wolff.

As discussed above, in response to the 112 and 103 rejections and the double-patenting rejection over '387, one would not have been motivated to combine the teachings of Milas with '616.

(viii) Claims appendix:

1. (previously presented) A process for delivering a polynucleotide to a skeletal muscle cell of a mammal in vivo, comprising:
   a) applying non-invasive external pressure against the skin of a limb of the mammal such that blood flow to and from the limb is impeded;
   b) inserting the polynucleotide encoding a protein operably linked to a promoter in a solution into a blood vessel in the limb in vivo distal to the applied pressure; and,
   c) administering immunosuppressive drugs to the mammal;

   thereby delivering the polynucleotide to the skeletal muscle cell in the limb distal to the applied pressure and expressing the polynucleotide in the skeletal muscle cell at detectable levels.
2. (original) The process of claim 1 wherein the polynucleotide consists of naked DNA.
3. (original) The process of claim 1 wherein the polynucleotide is selected from the group consisting of a viral vector and a non-viral vector.

4-5. (canceled)

6. (previously presented) The process of claim 1 wherein the limb skeletal muscle cell consists of a leg skeletal muscle cell.
7. (previously presented) The process of claim 1 wherein the limb skeletal muscle cell consists of an arm skeletal muscle cell.

8-10. (canceled)

11. (previously presented) The process of claim 7 wherein the arm skeletal muscle cell is selected from the group consisting of palmaris longus muscle cell, pronator teres muscle cell, flexor carpi radialis muscle cell, flexor carpi ulnaris muscle cell, and flexor digitorum superficialis muscle cell.
12. (previously presented) The process of claim 7 wherein the arm skeletal muscle cell is selected from the group consisting of flexor digitorum profundus muscle cell, and pronator quadratus muscle cell.

13-15. (canceled)

16. (previously presented) The process of claim 7 wherein the arm skeletal muscle cell is selected from the group consisting of brachioradialis muscle cell, extensor carpi radialis longus muscle cell, extensor carpi radialis brevis muscle cell, extensor digitorum muscle cell, anconeus muscle cell, extensor carpi ulnaris muscle cell, and extensor pollicis longus muscle cell.

17. (previously presented) The process of claim 7 wherein the arm skeletal muscle cell is selected from the group consisting of supinator muscle cell, abductor pollicis longus muscle cell, extensor digiti secund et teriti muscle cell, and extensor digiti quart et minimi muscle cell.
18. (previously presented) The process of claim 7 wherein the arm skeletal muscle cell consists of a hand skeletal muscle cell.
19. (previously presented) The process of claim 18 wherein the hand skeletal muscle cell consists of a thumb muscle cell.
20. (previously presented) The process of claim 18 wherein the hand skeletal muscle cell is consists of an interosseus muscle cell.

21-23. (canceled)

24. (previously presented) The process of claim 6 wherein the leg skeletal muscle cell is selected from the group consisting of gastrocnemius muscle cell and soleus muscle cell.
25. (previously presented) The process of claim 6 wherein the leg skeletal muscle cell is selected from the group consisting of popliteus muscle cell, flexor digitorum longus muscle cell, flexor hallucis longus muscle cell, and tibialis posterior muscle cell.

26-27. (canceled)

28. (previously presented) The process of claim 6 wherein the leg skeletal muscle cell consists of a foot skeletal muscle cell.
29. (previously presented) The process of claim 6 wherein the leg skeletal muscle cell is selected from the group consisting of tibialis anterior muscle cell, extensor hallucis longus muscle cell, extensor digitorum longus muscle cell, and abductor hallucis longus muscle cell.
30. (previously presented) The process of claim 6 wherein the leg skeletal muscle cell is selected from the group consisting of peronaus longus muscle cell and peronaus brevis muscle cell.
31. (previously presented) The process of claim 28 wherein the foot skeletal muscle cell is selected from the group consisting of extensor digitorum brevis muscle cell and extensor hallucis brevis muscle cell.

32-33. (canceled)

34. (previously presented) The process of claim 1 wherein applying non-invasive external pressure against the skin of the limb consists of applying a tourniquet around the limb.
35. (previously presented) The process of claim 1 wherein applying non-invasive external pressure against the skin of the limb consists of applying a cuff.

36. (previously presented) The process of claim 35 wherein the cuff consists of a sphygmomanometer.

37-38. (canceled)

39. (previously presented) A process for delivering a polynucleotide to a skeletal muscle cell of a mammal in vivo, comprising:

a) applying pressure non-invasively against the skin of the limb thereby impeding blood flow into and out of the limb;

b) inserting the polynucleotide encoding an expressible sequence operably linked to a promoter in a solution into a blood vessel in the limb of the mammal in vivo distal to the applied pressure;

c) delivering the polynucleotides to the skeletal muscle cell of the limb distal to the applied pressure; and,

d) expressing the polynucleotide in the skeletal muscle cell to detectable levels;

wherein said applying, said inserting, said delivering and said expressing do not diminish subsequent use of the limb by the mammal.

40. (previously presented) The process of claim 1 wherein administering immunosuppressive drugs consists of repetitive administration of immunosuppressive drugs.

41. (previously presented) The process of claim 1 wherein administering immunosuppressive drugs consists of administering immunosuprressive drugs within one day of injecting the polynucleotide.

42. (previously presented) The process of claim 1 wherein administering immunosuppressive drugs is selected from the group consisting of administering immunosuppressive drugs orally and administering immunosuppressive drugs by subcutaneous injection.

(ix) Evidence appendix

§1.132 Declarations that were entered during this prosecution are appended to this amended appeal brief along with the related supporting material.

Appended §1.132 Declaration 1 was entered on May 5, 2003.

Appended §1.132 Declaration 2 was entered on January 28, 2005

(x) Related proceedings appendix:

There are no decisions rendered by a court or the Board in any proceeding identified pursuant to paragraph (c)(1)(ii) of §41.37.

Pages 1-20 are
Respectfully resubmitted,

Mark K. Johnson Reg. No. 35,909
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I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as express mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on this date: __.

Mark K Johnson

DECLARATION 2 FOR AMENDED BRIEF

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : **09/707,117** Confirmation No. 8513
Applicants : **Jon A. Wolff, Vladimir Budker**
Filed : **11/06/2000**
Art Unit : **1632**
Examiner : **Wilson, Michael C.**
Docket No. : **Mirus.018.01**

For: **Intravascular Delivery of Nucleic Acid**

Commissioner of Patents
PO Box 1450
Alexandria, VA 2231-1450

DECLARATION UNDER 37 C.F.R. §1.132

Dear Sir:

I, Dr. Zane Neal, hereby declare as follows:

1. I have a Doctorate in Cellular/Molecular Immunology from the University of Wisconsin, Madison.
2. I am familiar with the above captioned application and the Budker et al. (1998, Gene Therapy, Vol. 5, pg 272-276) publication.
3. I am the author of the attached statement regarding the effect of collagenase on the immune system.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

/ZANE NEAL/ 1/27/2005

Dr. Zane Neal Date

DECLARATION 2 SUPPORT FOR AMENDED BRIEF

Re: Collagenase functioning as an immunosuppressive agent

In the patent application 09/707,117, the Action suggests that inclusion of collagenase in the invention for delivery of nucleic acids to skeletal muscle, may be considered to serve as an immunosuppressive agent. The Examiner is correct in interpreting that collagenase action affects the blood vessels by promoting increased permeability, transiently mimicking conditions similar to vascular leak syndrome or capillary leak syndrome. The Examiner's reasoning that diminished blood flow could impact immune function by decreasing trafficking of immune effector cells through the lymphatic system is unprecedented. A cursory review of the literature using Medline and PubMed failed to identify any scientific studies reporting such a phenomenon.

In my research experience understanding the immune response to infection and preclinical tumor immunobiology, I am unaware of employing increased vascular permeability as a method to induce locoregional or systemic immunosuppression. If loss in blood flow through the isolated limb during the delivery procedure was severe enough to negatively impact the immune status of the host by affecting the flow rate through the lymphatics, we would expect to observe signs of acute ischemia in the isolated limb.

Conversely, vascular damage and increased vascular permeability are more likely to induce immune activation by initiating immune inflammatory responses. The response to trauma begins in the immune system at the moment of injury. The loci are the wound, with activation of macrophages and production of proinflammatory mediators, and the microcirculation with activation of endothelial cells (EC), blood elements, and a capillary leak. These processes are potentiated by areas of micro-ischemia and impaired oxygen delivery and by the presence of necrotic tissue, each exacerbating the inflammatory response (Seminars in Pediatric Surgery 4:77-82, 1995). ECs are able to produce vasodilatory mediators and several factors lead to increased vascular permeability (i.e., similar to the effects of collagenase). ECs play a central role in immune cell extravasation, a key feature of inflammation (Rheumatic Diseases Clinics of North America 30:97-114, 2004). Unlike the patent reviewers position, I would anticipate use of collagenase to increase vascular permeability should promote an immediate inflammatory response and stimulation of immune system components.

Zane C. Neal, Ph.D.

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Madison WI, 53792

and

Senior Scientist-Immunology/Cancer Gene Therapy
Mirus Bio Corporation

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: **Jon A. Wolff, Vladimir G. Budker**

Serial No.: **09/707,117**

Filed: **November 6, 2000**

Group Art Unit: **1632**

Examiner: **Michael C. Wilson**

For: **Intravascular Delivery of Nucleic Acid**

DECLARATION UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, DC 20231

Dear Sir:

I, Jon A. Wolff, hereby declare as follows:

1. I am an inventor of the captioned application.

2. I submit with this Declaration and Response further experimental material (attached) illustrating *in vivo* delivery of nucleic acid-containing viral and non-viral vectors and unexpressed functional nucleic acid to nonvascular muscle cells. This material demonstrates a correlation between delivery of naked DNA, plasmid-containing non-viral complexes, viral complexes and functional non-expressed nucleic acid (siRNA). No new matter was used in the experiments.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

/JON A. WOLFF/ 5/9/2003
Jon A. Wolff Date

A. Delivery to Rat Skeletal Muscle cells In Vivo Using Intra-iliac Injection.
Delivery of negatively charged PEI/DNA and histone H1/DNA complexes to skeletal muscles in rat via a single injection into the iliac artery: PEI/DNA and histone H1/DNA particles were injected into rat leg muscle by a single intra-arterial injection into the external iliac. Female Harlan Sprague Dawley rats, approximately 150 g, each received complexes containing 100 μg plasmid DNA encoding the luciferase gene under control of the CMV enhancer/promoter (pCI-Luc) [Zhang et al. 1997]. Rat iliac injections of 10 mL of solution (n = 2) were conducted as previously described (see example 8 of patent application 09/707,117). Results of the rat injections are provided in relative light units (RLUs) and micrograms (μg) of luciferase produced. To determine RLUs, 10 μl of cell lysate were assayed luminometer and total muscle RLUs were determined by multiplying by the appropriate dilution factor. To determine the total amount of luciferase expressed per muscle we used a conversion equation that was determined in an earlier study [Zhang et al. 1997] [pg luciferase = RLUs x 5.1 x 10^{-5}].

Table 17. Luciferase expression in multiple muscles of the leg following injection of negatively charged DNA/PEI or DNA/Histone HI particles.

DNA/PEI particles (1 : 0.5 charge ratio)

Muscle Group	*Total RLUs*	*Total Luciferase*
muscle group 1 (upper leg anterior)	3.50×10^9	0.180 μg
muscle group 2 (upper leg posterior)	3.96×10^9	0.202 μg
muscle group 3 (upper leg medial)	7.20×10^9	0.368 μg
muscle group 4 (lower leg posterior)	9.90×10^9	0.505 μg
muscle group 5 (lower leg anterior)	9.47×10^8	0.048 μg
muscle group 6 (foot)	6.72×10^6	0.0003 μg

DNA/histone H1 particles (1 : 0.5 charge ratio)

Muscle Group	*Total RLUs*	*Total Luciferase*
muscle group 1 (upper leg anterior)	3.12×10^9	0.180 μg
muscle group 2 (upper leg posterior)	9.13×10^9	0.202 μg
muscle group 3 (upper leg medial)	1.23×10^{10}	0.368 μg
muscle group 4 (lower leg posterior)	5.73×10^9	0.505 μg
muscle group 5 (lower leg anterior)	4.81×10^8	0.048 μg
muscle group 6 (foot)	6.49×10^6	0.0003 μg

Results indicated delivery of the negatively charged complexes containing luciferase-expressing plasmid to muscles throughout the leg via injection into a afferent artery.

B. Adenoviral vectors can be delivered to muscle parenchymal cells by an intravascular route. An adenoviral vector CMVLacZ that expresses the E. coli β-galactosidase from the immediate early promoter of the human cytomegalovirus (CMV) was prepared as previously described (Yang et al. 1996) The rat iliac artery injection was performed as above. 0.5 mg of papaverine and 40 ng of collagenase in 3 ml saline was pre-injected while blocking the iliac artery and vein. 5×10^8 particles of the adenoviral vector CMVLacZ in 10 ml of saline was

injected in about 10 sec. After 2 minutes, the clamps were opened. Two days after injection, leg muscle cells were assayed for luciferase as above. Delivery was monitored be expression of luciferase encoded within the adenovirus genome. The results summarized in Table 24 demonstrate the delivery of Adenovirus to multiple muscle groups in the leg.

Table 24: Delivery of adenovirus expressing a luciferase gene to skeletal muscle via iliac injection.

Muscle Group	Luciferase (ng)
Upper Leg Anterior	59.04
Upper Leg Posterior	18.33
Upper Leg Medial	4.44
Lower Leg Posterior	11.04
Lower Leg Anterior	5.33
Foot	0.22
Total	98.40

C. Delivery of siRNA to muscle cells in rat via an intra-iliac administration route: 10 μg pGL3 control and 1 μg pRL-SV40 with 5.0 μg siRNA-luc+ or 5.0 siRNA-ori were injected into iliac artery of rats. Specifically, animals were anesthetized and the surgical field shaved and prepped with an antiseptic. The animals were placed on a heating pad to prevent loss of body heat during the surgical procedure. A midline abdominal incision was made after which skin flaps were folded away and held with clamps to expose the target area. A moist gauze was applied to prevent excessive drying of internal organs. Intestines were moved to visualize the iliac veins and arteries. Microvessel clips were placed on the external iliac, caudal epigastric, internal iliac, deferent duct, and gluteal arteries and veins to block both outflow and inflow of the blood to the leg. An efflux enhancer solution (e.g., 0.5 mg papaverine in 3 ml saline) was injected into the external iliac artery though a 25 g needle, followed by the plasmid DNA and siRNA containing solution (in 10 ml saline) 1-10 minutes later. The solution was injected in approximately 10 seconds. The microvessel clips were removed 2 minutes after the injection and bleeding was controlled with pressure and gel foam. The abdominal muscles and skin were closed with 4-0 dexon suture.

Four days after injection, rats were sacrificed and the quadriceps and gastrocnemius muscles were harvested and homogenized. Luc+ and Renilla Luc activities were assayed using the Dual Luciferase Reporter Assay System (Promega). Ratios of Luc+ to Renilla Luc were normalized to the siRNA-ori control. siRNA-Luc+ inhibited Luc+ expression in quadriceps and gastrocnemius by 85% and 92%, respectively, compared to the control siRNA-ori. Thus siRNA was effectively delivered to muscle cells in the leg using the delivery procedure.